

# Honey Bee-Infecting Plant Virus with Implications on Honey Bee Colony Health

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**ABSTRACT** Honey bees are eusocial insects that are commercially managed to provide pollination services for agricultural crops. Recent increased losses of honey bee colonies (averaging 32% annually since 2006) are associated with the incidence and abundance of pathogens. In their study in *mBio*, J. L. Li et al. [mBio 5(1):e00898-13, 2014, doi:10.1128/mBio.00898-13] share their discovery that a plant virus, tobacco ring spot virus (TRSV), replicates in honey bees and that the prevalence of this virus was high in weak colonies. Their findings increase our understanding of the role of viruses in honey bee colony losses and underscore the importance of surveying for new and/or emerging viruses in honey bees. Furthermore, their findings will pique the interest of virologists and biologists across all disciplines. The discovery that a plant virus (TRSV) replicates, spreads, and negatively affects the health of an insect host will lead to additional studies on the mechanisms of host-specific adaptation and the role of cross-kingdom infections in the transmission of this virus.

Honey bees (*Apis mellifera*) pollinate numerous agricultural crops, including almonds, apples, blueberries, and oil seed crops, valued at a total of \$14.6 billion annually (1). They are also responsible for pollination of plant species that augment the biodiversity of agricultural and nonagricultural landscapes. Increased annual losses of honey bee colonies in the United States, Canada, and Europe have alarmed agricultural specialists, beekeepers, growers, scientists, and the general public. These losses are in part due to a phenomenon known as colony collapse disorder (CCD), which occurs predominantly over the fall/winter months. The cause(s) of increased colony losses remain unknown, but the current prevailing theory is that multiple factors (i.e., pathogens, poor bee nutrition, and agrochemical exposure) acting in concert can reduce colony longevity (2, 3). While no specific pathogen(s) has been shown to be consistently associated with CCD and/or overwinter losses, pathogen incidence and abundance correlate with colony loss (2, 4). Therefore, continued efforts to monitor and discover bee pathogens are extremely important to agriculture and global food production. Furthermore, these research efforts lead to extremely interesting biological findings, including those of Li et al., as reported recently in this journal (5).

Li et al. discovered that a plant virus, tobacco ring spot virus (TRSV), infects and replicates in honey bees. This is a fascinating example of an RNA virus that can infect both insect and plant hosts. Furthermore, Li et al. determined that weak honey bee colonies, defined by low colony population size and collapse, had a higher prevalence of TRSV than healthy colonies (5). These findings expand the realm of pathogens that may play important roles in honey bee health.

The majority of honey bee viruses are positive-sense, single-stranded RNA (ssRNA) viruses in the order *Picornavirales* (reviewed in reference 6). Taxonomic classification, as inferred from phylogenetic analyses of virus-encoded RNA-dependent RNA polymerases (RdRp), further delineates picorna-like viruses into the *Picornaviridae*, *Dicistroviridae*, and *Iflaviridae* families (7). Common honey bee viruses in the *Dicistroviridae* family include acute bee paralysis virus (ABPV), black queen cell virus (BQCV), Israeli acute paralysis virus (IAPV), and Kashmir bee virus (KBV). Iflaviruses include Sacbrood virus (SBV), deformed wing virus (DWV), and slow bee paralysis virus (SBPV). Additional viruses,

including chronic bee paralysis virus (CBPV), Kakugo virus, and the Lake Sinai viruses (LSVs), remain unclassified (8–10). Virus infections in honey bees can be asymptomatic, cause deformities and/or paralysis, or result in death.

Advances in molecular techniques, including genome sequencing, PCR-mediated detection, microarray-based detection and discovery, and ultra-high-throughput (or next-generation) sequencing have increased our understanding of virus prevalence, phylogenetic relatedness, and association with colony health (4, 6, 10–14). Recently, next-generation sequencing of honey bee-associated viruses determined the prevalence of Israeli acute paralysis virus in samples from CCD-affected bees (12), identified a new honey bee-associated strain of aphid lethal paralysis virus (ALPV; strain Brookings) (10, 15), and resulted in the discovery of a new group of honey bee-infecting viruses, the LSVs (10), which have been associated with CCD in the United States (4) and overwinter losses in Belgium (16). These recent discoveries, together with those of Li et al., underscore the importance of research aimed at discovering, detecting, and characterizing the pathogenesis, phylogenetic relatedness, and geographic distribution of honey bee viruses, as well as the investigation of these pathogens in the context of the entire honey bee microbiome (17–19).

Like the majority of honey bee viruses, TRSV is a positive-sense ssRNA virus within the *Picornavirales* order. TRSV was first observed in infected tobacco and is known to infect numerous plant species, including crops, weeds, and ornamentals. TRSV is vectored by nematodes and insects (e.g., aphids, thrips, grasshoppers, and the tobacco flea beetle). The work by Li et al. significantly expands the known host range of TRSV to include honey bees. The phylogenetic relationship between common honey bee viruses and TRSV inferred from the RdRp sequence is well illustrated in the work of Koonin et al. (7), which includes honey bee viruses on

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two branches and TRSV on the third branch of a large virus clade composed of chromalveolate-, plant-, and arthropod-infecting viruses.

Interestingly, the majority of viruses transmitted by insects to animal hosts (i.e., arboviruses) replicate within insect vectors, whereas the majority of insect-transmitted plant viruses do not replicate in insect vectors (20). Therefore, the finding of Li et al. that TRSV replicates in honey bees is particularly fascinating. Furthermore, the distribution of TRSV in honey bees differs from that seen in insects that serve as vectors primarily for plant viruses. Specifically, TRSV was detected throughout the honey bee body, whereas plant viruses that replicate in aphid and thrip vectors are found primarily in the gut and salivary gland. Li et al. demonstrated that the *Varroa destructor* mite, an ectoparasite of honey bees that transmits some honey bee viruses, also harbors TRSV. The distribution pattern of TRSV in the *Varroa destructor* mite, as demonstrated by *in situ* hybridization, is similar to that of arthropod viral vectors. Future studies that determine whether TRSV replicates in *Varroa*, and whether it indeed transmits TRSV to honey bees, are important for understanding the transmission dynamics of this newly described honey bee pathogen.

The phylogenetic relationship among the honey bee, *Varroa* mite, and pollen-associated TRSV isolates described by Li et al., as well as plant TRSV isolates in the NCBI database, was inferred from a 731-nucleotide, capsid protein-encoding region of the genome. The resulting phylogenetic tree indicated that the bee, mite, and pollen isolates are indistinguishable from each other but distinct from the plant isolates. Full-length genome sequences of TRSV isolates are needed to address several questions, including the following. Are there host-specific TRSV mutations, particularly in viral proteins that interact with the host (e.g., capsid proteins)? Do TRSVs isolated from plants have increased conservation in the genome region encoding the movement protein (MP), which facilitates virus passage through plant plasmodesmata? Will the TRSV VPg (virus protein, genome-linked) genome region in plant and bee isolates diverge over time? Does this virus regularly shuttle between plant and insect hosts, and if so, will host-specific mutations be difficult to confirm? Comparative analyses of TRSV isolates from historic samples and currently circulating isolates from diverse geographic regions may be used to begin to address some of these exciting, biologically relevant questions. Likewise, additional studies aimed at assessing the host range of TRSV in other insects (e.g., bees, wasps, and ants) will further our understanding of the interspecies transmission.

Clearly the findings by Li et al. will excite scientists from all fields, since the discovery that a plant virus (TRSV) infects and replicates in honey bees is a unique finding with broad implications. The observation that TRSV infection correlates with poor colony health and colony collapse warrants further investigation and may result in a better understanding of increased honey bee losses. This work is particularly relevant for researchers examining correlations between individual bee and colony health with pathogen- and host-associated microbial profiles. Poor colony health and colony losses are associated with increased pathogen incidence and abundance. Since the majority of honey bee pathogens are RNA viruses, it is likely that RNA interference (RNAi)-mediated antiviral strategies are employed by honey bees to control viral invasion (21–23), much like fruit flies and mosquitoes (reviewed in references 24 and 25). However, basic questions regarding the mechanism(s) of honey bee antiviral immunity re-

main unanswered. For example, our studies demonstrate that double-stranded RNA (dsRNA) of any specificity decreases virus load in honey bees, which indicates the potential of additional dsRNA-triggered immune mechanisms (26); likewise, other studies demonstrate unexpected off-target effects of dsRNA (27). Together, these studies suggest that antiviral defense in honey bees involves RNAi and additional dsRNA-triggered antiviral immune pathways. Future studies are needed to determine the molecular mechanisms and relative importance of these responses in specific bee-virus interactions.

As Li et al. have demonstrated, basic science often leads to the discovery of the unexpected. We anticipate that future experiments in this field will lead to the discovery of additional bee pathogens and reveal the mechanisms of honey bee antiviral immunity. These studies will result in a greater understanding of the impact of viruses on honey bee health. Such discoveries may redefine our understanding of honey bee health, immunity, and disease transmission and are paramount to understanding the current crisis facing honey bees.

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